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Side Effects From Dermatological Laser Treatment Related to UV Exposure and Epidermal Thickness: A Murine Experiment With the Copper Vapor Laser

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Background and Objective: **The intention of the present study was to clarify whether UV-exposure prior to laser treatment of albino mouse skin may influence laser-induced side effects and if a possible influence may be explained by epidermal thickening.**
Study Design/Materials and Methods: **Albino, hairless mice were irradiated 0, 8, and 22 consecutive times with simulated solar UV (1.4 J/cm^2 per treatment). Subsequently, two areas on the back of the mice (1.26 cm^2 each) were treated with a copper vapor laser that was connected to a Hexascan. The beam diameter was 1 mm, pulse duration 200 msec, and intensities 0.6 W, 0.8 W, and 1.0 W. Laser-induced wounds, scars, and histologically evaluated fibrosis were evaluated.**
Results: **We found that mice irradiated with UV before laser treatment developed smaller wounds, smaller texture change areas, and less fibrosis as compared with nonirradiated control groups, and significant, negative correlations were demonstrated between epidermal thicknesses (stratum corneum, the cellular part of epidermis, and the entire epidermis) and laser-induced skin reactions. A dose response was obtained between laser intensities and laser-induced skin reactions, which tended to be more severe in the cranial back location as compared with the caudal back location. Epidermal layers increased significantly after eight consecutive times of UV irradiation and increased to a steady level after 22 times of irradiation.**
Conclusion: **We conclude that UV exposure prior to laser treatment of albino mice reduced laser-induced side-effects, which could be explained by increased epidermal thickening. Variations in epidermal thickness might thus contribute to variations in clinical response to dermatological laser treatment with the copper vapor laser. Lasers Surg. Medicine 20:233-241, 1997.**

Key words: cicatrix; laser surgery; UV radiation; wound healing

INTRODUCTION

Port wine stained lesions (PWS) are congenital malformations, characterized by multiple dilated vessels in the dermis. These vessels show increasing ectasia with age, which results in a change in color from initially light pink to dark purple, just as nodularities may develop [1]. Thetreatment of PWS and other vascular lesions has

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been revolutionized by the development of lasers, which emit yellow light (dye laser 577 nm, 585 nm, and copper vapor laser (CVL), 578 nm), since the wavelengths of these lasers match the oxyhemoglobin absorption curve and deliver their energy very precisely to the dermal vessels [2–5]. The pulsed dye laser, which meets the requirements of selective photothermolysis (585 nm, 450 μsec), has become widely used and is especially preferred for treatment of young patients with macular, faint lesions [6,7], whereas the CVL, which operates in a quasicontinuous mode (578 nm, 50–200 msec), has been advocated for treatment of older patients with dark, thick PWS, having a large vessel diameter, since pulse durations in the msec range allow for extended thermal diffusion and thereby for destruction of vessel walls of larger calibre vessels [8–10].

Clinical experience and animal studies indicate that the end result from laser treatment of benign, vascular malformations may be suboptimal, if the treated skin area is exposed to solar ultraviolet (solar UV) radiation, which may intensify hyperpigmentation and scarring [2,11]. Therefore, laser surgeons recommend avoidance of sun exposure following laser treatment for 3 months, mainly in order to minimize the chances of hyperpigmentation [2,12]. Moreover, sun exposure *prior* to laser treatment is known to increase skin pigmentation as well as epidermal thickness [13]. Skin pigmentation is of importance to the vascular selectivity and the final outcome from laser exposure, since melanin represents an overlaying, competitive chromophore, through which the laser light must pass in order to reach the intravascular oxyhemoglobin [14,15]. However, as far as we know, the impact of variations in epidermal thickness on the final end result of treatment of vascular lesions has not been investigated. Variations in epidermal thickness may be of importance to patients exposed to sunlight prior to laser treatment, as well as variations in epidermal thickness might contribute to an explanation of age- and anatomical differences in response to laser treatment. The increased epidermal thickness may thus inhibit penetration of laser light and leave deeper located vessels incapable of being targeted, resulting in a suboptimal end result [14,16–18].

The intention of the present study was therefore to detect if exposure to solar UV prior to laser treatment of albino mouse skin may influence laser-induced side effects and if a possible influence may be explained by epidermal thickening.

MATERIALS AND METHODS

Animals

Hairless, albino, female MORO/Ibm—hr/hr, barrier bred mice (n = 188, Biological Research Laboratories, Basel, Switzerland; Bomholt Breeding and Research Center, Denmark) were randomized into 12 groups (Table 1). The individual mice were tattooed with consecutive numbers on the abdomen. The experiment began after 6 weeks of acclimatization, when the animals were 13–18 weeks old. The animals had free access to water and standard laboratory chow and were kept on a 12-h light/dark cycle. The room temperature at the animal facilities was kept between 23°C and 24°C. The guidelines for humane treatment of animals were approved by the institution's animal use committee and by the Ministry of Justice.

Experimental Design

UV irradiation was performed on 0, 8, and 22 consecutive days prior to laser treatment in order to induce varying thicknesses of stratum corneum, the cellular part of epidermis, and of the entire epidermis (Table 1). Every UV exposure was below the erythema level for the mice. However, the cumulative effect of the daily successive UV exposures occasionally induced erythema. A period of 96 hr ±4 hr passed between the last UV irradiation and the laser treatment in order to eliminate UV-induced acute inflammation. Laser treatments were performed on the midline dorsal skin of 150 mice in one or two identically treated areas, which were separated by 1–1.5 cm in order to avoid interference. Superficial biopsies were taken immediately prior to laser treatment for measurement of thicknesses of stratum corneum and the cellular part of epidermis. The mice were anaesthetized by intraperitoneal injections of fluanisone, fentanyl and midazolam prior to biopsies and laser treatment. During treatment, the mice were stretched in order to smooth out their skin and to flatten their backs, and three ink spots were tattooed on the back, functioning as fixpoints for the laser application. Thereby, it was possible to relocate the laser-treated areas independently of visible scarring. In the laser surgery room, the temperature was between 26°C and 28°C. At the 29th day after the treatment, the mice were sacrificed in CO_2 atmosphere, and two 4-mm punch biopsies were taken from the centre of the two laser-treated areas of each animal for histologic evaluation of fibrosis.

TABLE I. Treatment Schedule for 12 Groups with Total of 188 Mice and Distribution of Mice with Degree of Histologically Evaluated Fibrosis*

Treatment	No. mice	Mice with degree of fibrosis 0	1	2	3	Median
0 W	15	10/10				0/0
8 UV + 0 W	11	7/10				0/0
22 UV + 0 W	12	7/9	2/1			0/0
0.6 W	15	3/8	7/2			1/0
8 UV + 0.6 W	16	10/10				0/0
22 UV + 0.6 W	18	10/11	3/2			0/0
0.8 W	17	0/1	0/2	1/3	9/4	3/2
8 UV + 0.8 W	18		0/2	4/7	4/1	2.5/2
22 UV + 0.8 W	18	0/1	3/4	5/4	1/1	2/1.5
1.0 W	17			0/2	10/8	3/3
8 UV + 1.0 W	15			0/1	5/9	3/3
22 UV + 1.0 W	16			2/6	3/4	3/2
12 groups	188 mice					

*Scores of fibrosis from the cranial treated areas are depicted in front of the slash (total no of evaluations = 106); scores from the caudal treated area behind the slash (total no of evaluations = 123). Median values of histological scores from cranial and caudal locations are shown for treated groups (right column).

Laser Techniques

A CVL, PBI MultiLase D, (PBI Medical, Denmark) was used in connection with a microprocessor controlled handpiece, a Hexascan device (Prein & Partners, Ferney, Voltaire, France). The laser pulses were applied to the skin from a fixed distance and in a fixed jumping mode with the purpose of minimizing thermal diffusion and obtaining a high degree of uniformity of the treated areas. The CVL operates in a quasicontinuous mode, producing a rapid train of pulses and emitting 8,400 pulses/sec (8.4 kHz) at 578 nm yellow band. Beam diameter was 1 mm, pulse duration 250 msec, and the total exposure area was 1.26 cm^2 consisting of 127 pulses. The intensities used were 0.6, 0.8, and 1.0 W/spot, corresponding to calibrated Hexascan-fluences of 19.1, 25.5, and 31.8 J/cm^2. Laser intensities were controlled by a power-meter (Analogue Power Read Out no. 25 APR. Power Meter Head no. 25 V-VIS, Photon Control).

UV Radiation Sources

Simulated solar UV radiation was obtained from a bank of tubes consisting of 1 Phillips TL 12 and 5 Bellarium-S SA-1-12 tubes. The emission spectrum of the radiation source ranged between 280 nm and 400 nm, with maximum peak between 340 and 350 nm. The emission spectrum was measured in 1 nm steps using a Jobin Yvon monochromator H10 double UV (slit widths 0.5, 1.0, and 0.5 mm) with a selective mirror blocking out visible light and an International Light (IL) 1700 research radiometer with a SHD 033 detector. The monochromator was calibrated with an Optronic Laboratories deuterium lamp precision source model 45D. The intensity of the UV-source was measured with the IL 1700 research radiometer with an IL SED 400 detector, a WBS 320 filter, and a quartz diffuser. Measurements were performed under the grid of the cage at mouse back level. The intensities were corrected on basis of the spectral sensitivities of the detectors and the emission spectrum of the source. Exposure doses in Basic-Minimal Erythema Doses (B-MEDs) were derived from the erythema effectiveness spectrum, the CIE erythema action spectrum of McKinlay and Diffey [19], and taking as reference for one B-MED a 24-hr MED at 296 nm of 31.2 mJ/cm^2 [20]. The daily UV dose of 1.4 J/cm^2 was equivalent to 2.3 B-MED. The cumulative UV-dose was 11.2 J/cm^2 for groups irradiated eight times and 30.8 J/cm^2 for groups irradiated 22 times.

Macroscopic Evaluation

The wounds were examined every second day by drawing their outline on a mm paper in order to quantify the *maximum size* of the wounds. Registration continued until the wounds were completely covered with epidermis and no eschars were left. Subsequently, we evaluated the maximum size of area with texture change by the same method, and the presence of atrophy, infiltration, and contraction was registered at the end of the experiment. *Texture change* was defined as any visual or palpable change of skin appearance, *atrophy* as visual or palpable depression of skin texture, and *infiltration* as visual or palpable increased skin consistency. All measurements were made by one person to assure consistency.

Light Microscopy

LM was used to measure the thickness of stratum corneum and of the cellular part of epidermis and to assess the degree of laser-induced fibrosis as described below:

Thicknesses of stratum corneum and the cellular part of epidermis. Superficial biopsies (n = 94) were taken in triplicate immediately prior to laser treatment from mice treated with 0.6 W. The biopsies (4 mm^2) were taken from the midline dorsal skin close to the two laser-treated

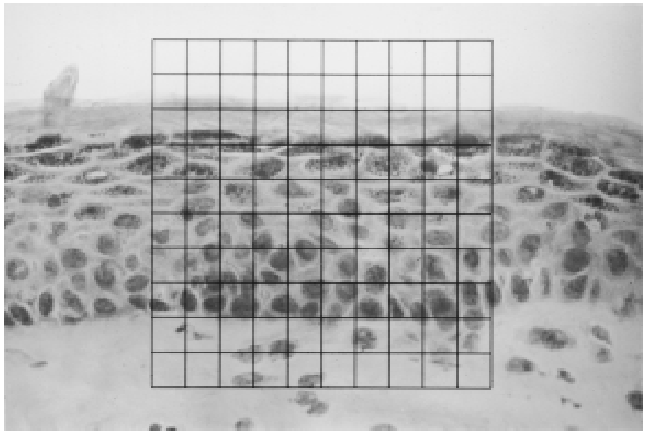

Fig. 1. Histology of albino mouse epidermis irradiated daily for 22 consecutive days with an accumulated UV doses of 30.8 J/cm^2. Cell proliferation is seen, which results in increased thicknesses of stratum corneum, the cellular part of epidermis, and consequently of the entire epidermis. The square grid is used for calculating the epithelial height of the cellular part of epidermis. The number of crossover points inside the cellular part of epidermis was counted and expressed the area, the length of the grid was held constant, and subsequently the epithelial height could be calculated (area = length × height). The height expressed in counting numbers was converted into exact epithelial height in micrometers by adjusting for the used optics, which was calibrated before utilization.

areas, but not close enough to cause interference with the laser treatment. Specimens were snap-frozen, fixed in pentane, and stored at –80°C. Sections were cut at 8 μm and stained with haematoxylin and eosin. The slice of highest technical quality out of 5–10 slices from one biopsy was evaluated, and at least four evaluations were performed per slice with the purpose of obtaining representative mean values. Measuring the thickness of the *cellular part of epidermis* was based on an area measurement by means of a square grid (Fig. 1). *Stratum corneum* was measured by means of a ruler that was placed perpendicular to the basis of the epithelium. The measured value was adjusted for magnification optics and expressed the height of stratum corneum directly in micrometers. *Thickness of the entire epidermis* was assessed by adding thicknesses of stratum corneum and the cellular part of epidermis. Calculated thicknesses in the laser treated areas of stratum corneum, of the cellular part of epidermis, and of the entire epidermis were calculated as average values for two adjacent biopsies.

Quantification of fibrosis. Formalin fixed biopsies were used for semiquantitative evaluation of fibrosis that was divided arbitrarily into four grades (0-1-2-3). Staining procedures included hematoxylin-eosin and van Gieson-Hansen. Grade 0: no evident fibrosis, normal skin. Grade 1: just recognizable increase in the quantity of fibrosis, localized to the superficial part of dermis. Grade 2: changes between grade 1 and grade 3. The cuticula musculature is not involved in the fibrosis. Grade 3: massive fibrosis, localized to the entire layer of dermis with the cuticula musculature being partly or totally fibrosed. The profound epidermal layer is flattened, corresponding to disappearance of the epidermal taps and the dermal papillae. The accessory skin structures are diminished.

Statistics. The statistical analyses were performed by means of nonparametric methods: Kruskal Wallis test, Mann-Whitney U test, Wilcoxon test, and Spearmann rank correlation coefficient. Differences between the groups were considered significant when $P \leqslant 0.05$.

RESULTS

Results are presented on (1) acute skin reactions, (2) chronic side-effects from laser treatment of mouse skin with UV-induced epidermal thickening, and (3) the relation between epidermal thickness and laser-induced skin reactions. Acute parameters derived from maximum wound size; chronic parameters from maximum size of texture changes and from histologically evaluated fibrosis.

Acute Skin Reactions

Wound area. The maximum size of the wounds developed within 5 days and nights after the laser treatment. From Figure 2 it is observed that maximum wound areas turned smaller when laser-treated skin was pretreated with increasing UV doses. At the cranial location, 8 and 22 UV-preirradiations resulted in significantly smaller wounds as compared with nonirradiated mice at 0.6 and 0.8 W ($P < 0.02$); at the caudal location 22 UV irradiations prior to laser treatment with 1.0 W resulted in significantly smaller wounds as compared with the non-UV-irradiated group ($P < 0.05$); no other differences were significant within the same laser intensities. A dose response was obtained between the used laser intensities and the maximum wound areas within groups preirradiated with 0, 8, and 22 times of UV (0 UV+0.6 W vs. 0 UV+0.8 W vs. 0 UV+1.0 W; 8 UV+

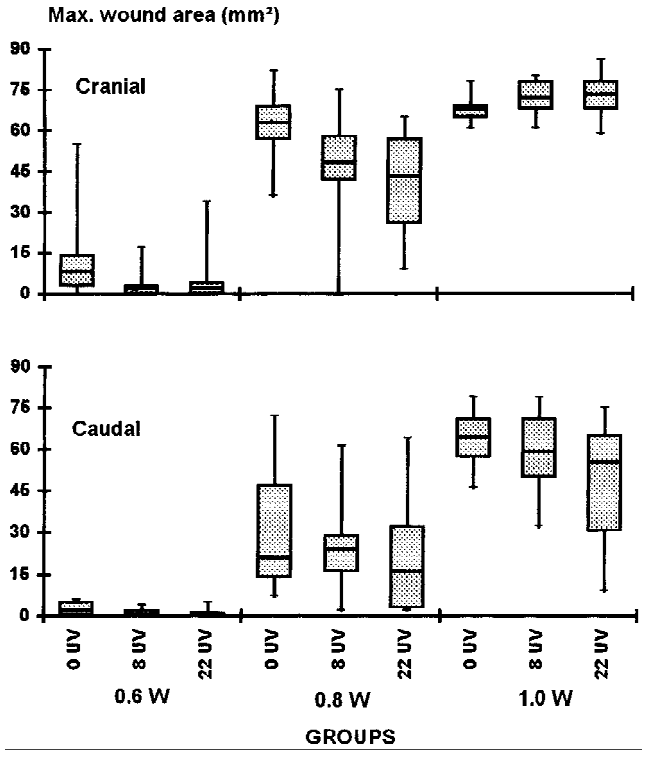

Fig. 2. Maximum wound area (mm^2) illustrated for two anatomical locations; cranial and caudal. Groups were pretreated with no UV, 8 times of UV, or 22 times of UV, followed by laser treatment with 0.6 W, 0.8 W, and 1.0 W laser intensity. The box extends from the 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile). Whiskers show the range of the data.

0.6 W vs. 8 UV+0.8 W vs. 8 UV+1.0 W; 22 UV+ 0.6 W vs. 22 UV+0.8 W vs. 22 UV+1.0 W; differences were significant at both anatomical locations between all laser intensities ($P<0.005$); except at the cranial location between groups treated with 0 UV+0.8 W vs. 0 UV+1.0 W). Wounds were significantly larger at the cranial location as compared with the caudal location in all except two groups (0 UV+1.0 W and 8 UV+0.6 W treated groups).

Chronic Side-Effects

Macroscopic scar evaluation. Gross skin reactions showed a spectrum from no visible changes in the groups treated with the lowest laser intensities to atrophic scars, which could be surrounded by a border zone of visual or palpable infiltration: Atrophy was observed in the group treated with 0.6 W laser intensity without UV preirradiation at cranial and caudal locations, whereas mice irradiated with 22 times of UV before 0.6 W laser treatment did not develop any macroscopically visible skin reactions. Atrophy and infiltration was observed at both the cranial and caudal treated areas in all groups treated with 0.8 W and 1.0 W. No contraction occurred in any mice.

Maximum size of area with texture change. In Figure 3, areas with maximum texture changes are depicted for two anatomical locations. Within groups treated with 0.6 W, we found that texture change areas decreased significantly when UV pretreatment was performed (0 UV+0.6 W vs. 8 UV+0.6 W vs. 22 UV+0.6 W, cranial and caudal locations; $P<0.05$). Within groups treated with either 0.8 W or 1.0 W, only two significant differences were found (0 UV+0.8 W vs. 22 UV+0.8 W, and 8 UV+0.8 W vs. 22 UV+0.8 W, caudal locations; $P<0.05$). On the whole, a dose response was obtained between laser intensities and maximum texture change areas within laser-treated groups, preirradiated with 0, 8, or 22 times of UV. Differences were significant at both cranial and caudal locations between 0.6 W and 0.8 W (0 UV+0.6 W vs. 0 UV+0.8 W, 8 UV+0.6 W vs. 8 UV+0.8 W, 22 UV+0.6 W vs. 22 UV+0.8 W; $P<0.01$), whereas no differences, except one, were significant from 0.8 to 1.0 W (22 UV+0.8 W vs. 22 UV+1.0 W, caudal location; $P<0.05$). We found that areas with texture changes were significantly larger at the cranial location as compared with the caudal location in laser treated groups that were preirradiated with 0 and 22 times of UV before laser treatment (0 UV+0.6 W, 0 UV+0.8 W, 0 UV+ 1.0 W, 22 UV+0.6 W, 22 UV+0.8 W, 22 UV+1.0 W; $P<0.05$), whereas no significant differences were found within groups irradiated with eight times of UV before laser treatment (8 UV+0.6 W, 8 UV+0.8 W, 8 UV+1.0 W).

Fibrosis. Table 1 shows the distribution of mice with degrees of fibrosis at cranial and caudal laser treated, dorsal skin areas. Within the same laser intensities, groups irradiated with UV before laser treatment tended to develop lower scores of fibrosis as compared with the corresponding laser treated control groups. At the cranial location this could be significantly confirmed for mice treated with 0.6 and 0.8 W (8 UV+0.6 W vs. 0 UV+0.6 W, 22 UV+0.6 W vs. 0 UV+0.6 W, and 22 UV+0.8 W vs. 0 UV+0.8 W; $P<0.01$). At the caudal location no differences were significant within the same laser intensity. Comparing scores of fibrosis in the two anatomical locations, it turned out that the cranial location tended to score higher values as compared with the caudal

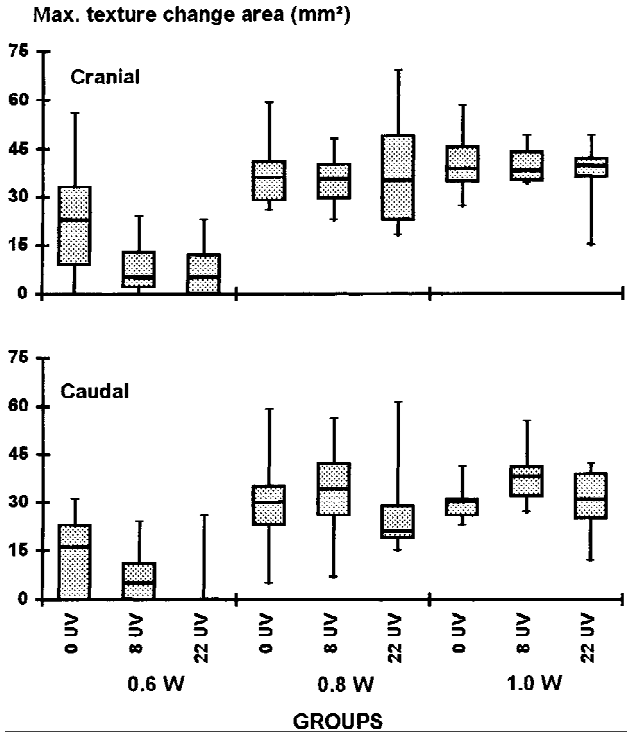

Fig. 3. Maximum texture change area (mm^2) illustrated for two anatomical locations; cranial and caudal. Groups were pretreated with no UV, 8 times of UV, or 22 times of UV, followed by laser treatment with 0.6 W, 0.8 W, and 1.0 W laser intensity. See Figure 2 for explanation of box and whiskers.

location. However, this was only significant in the 0.8 W laser treated group ($P<0.05$).

Relation Between Epidermal Thickness and Laser-Induced Skin Reactions

Epidermal thickness. From Figure 4, it is seen that thicknesses of *stratum corneum, the cellular part of epidermis,* and the *entire epidermis* increased significantly, when UV irradiation was performed eight times as compared with nonirradiated groups (cranial and caudal locations; $P<0.001$). Extension of UV irradiation from 8 to 22 times resulted in additional, but nonsignificant increases for the entire epidermis and the cellular part of epidermis, whereas the increase was significant for stratum corneum at the cranial location ($P<0.02$). Mice that were UV-irradiated 0 and 22 times had similar thicknesses in the cranial and caudal back locations, whereas mice irradiated eight times with UV obtained a significantly higher thickness at the caudal location as compared with at the cranial location ($P<0.01$;

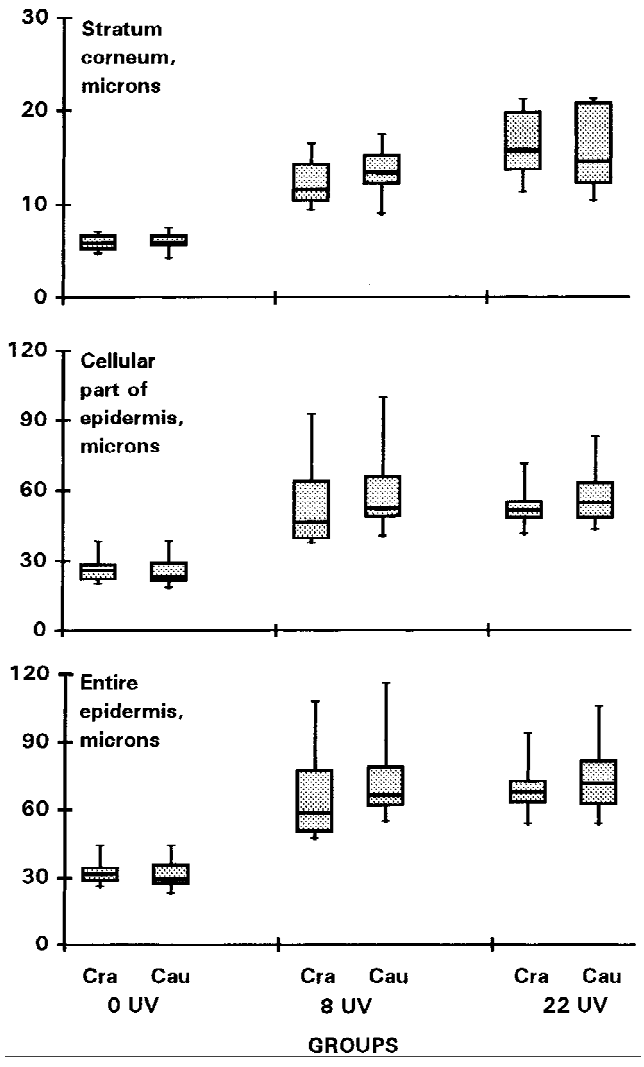

Fig. 4. Thicknesses of stratum corneum, the cellular part of epidermis, and the entire epidermis (μm) are illustrated for groups pretreated with no UV, 8 times of UV, and 22 times of UV. "Cra" and "cau" refers to the two midline dorsal anatomical locations; respectively, cranial and caudal. See Figure 2 for explanation of box and whiskers.

stratum corneum, the cellular part of epidermis, and the entire epidermis).

Correlation among epidermal thickness, acute skin reactions, and chronic side effects. We depicted maximum wound area, maximum texture change area, and score of fibrosis versus the entire epidermal thickness as illustrated in Figure 5, and negative, significant correlations were found. Maximum wound area, maximum texture change area, and score of fibrosis had similar appearances with negative, significant correlations when depicted versus stratum

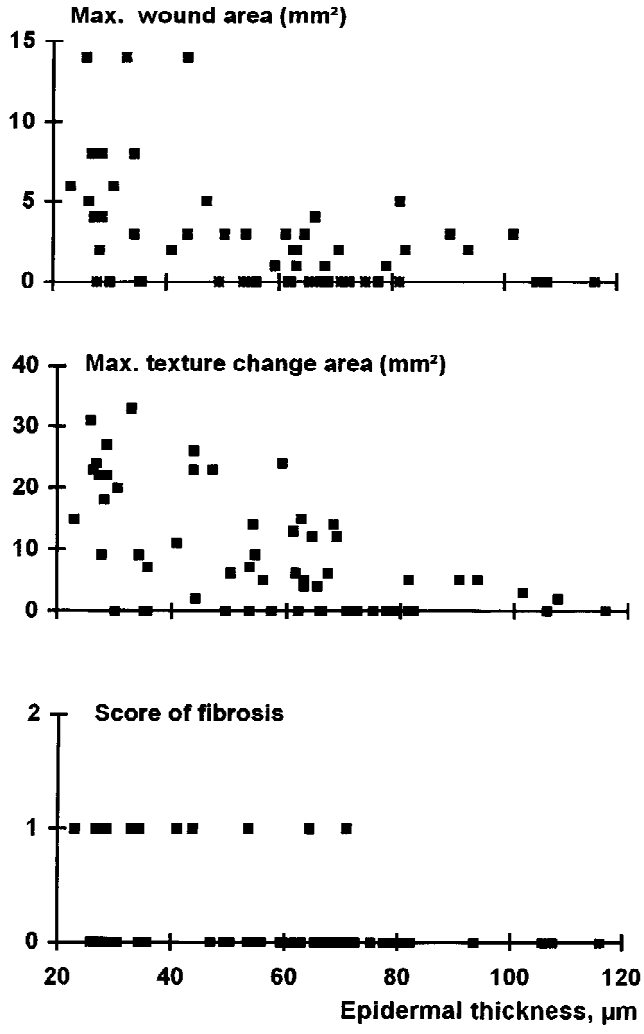

Fig. 5. Maximum wound area (mm^2), maximum texture change area (mm^2), and score of fibrosis versus thickness of the entire epidermis (μm). Each point refers to one mouse treated with 0.6 W laser intensity, and values for both cranial and caudal locations are depicted. The data points located on the X axis at Y = 0 represent no laser-induced wounds, texture changes, or fibrosis. For the *maximum wound area,* Spearmann correlation coefficient and P values were: 0.42, $P \leq 0.0008$ (entire epidermis); –0.50, $P \leq 0.0001$ (stratum corneum); and –0.38, $P \leq 0.002$ (cellular part of epidermis:). For the *maximum texture change area,* Spearmann correlation coefficients were –0.59, $P < 0.0001$ (entire epidermis); –0.64, $P < 0.0001$ (stratum corneum); and –0.59, $P < 0.0001$ (cellular part of epidermis). Concerning *score of fibrosis,* Spearmann correlation coefficients were –0.36, $P \leq 0.006$ (entire epidermis); –0.39, $P = 0.002$ (stratum corneum); and –0.35, $P \leq 0.007$ (cellular part of epidermis).

corneum and versus the cellular part of epidermis (not illustrated). Correlation coefficients and P values are stated in Figure 5.

DISCUSSION

It is well known that UV-B irradiation of hairless mice induces epidermal hyperplasia, which results in increased epidermal thickness [21]. This knowledge has constituted the basis of our study in which we have laser-treated albino hairless mice with different UV-induced epidermal thicknesses. We found that mice irradiated with UV before laser treatment developed less severe skin reactions than mice, which received the same laser treatment but no UV irradiation. Moreover, we did find significant, negative lations between epidermal thicknesses (stratum corneum, cellular part of epidermis, and entire epidermis) and laser-induced skin reactions. The strongest negative correlations (closest to –1) were observed for stratum corneum, which indicates that the best monotonous relation occurred between stratum corneum and laser-induced skin reactions. However, we decided in our graphic scatter plots (Fig. 5) to illustrate the entire epidermal thickness, since all epidermal layers contribute to attenuation of laser light. Stratum corneum thickness was found to constitute between 11% and 29% of the entire epidermis (mean value 20%), which is in accordance with previously reported results by Sterenborg et al. [21] where stratum corneum was found to constitute 22% of the whole epidermis. Furthermore, we did find that skin reactions in the cranial location tended to be more severe as compared with skin reactions in the caudal location. This might partially be explained by a smaller epidemal thickness in the cranial anatomical location, since we found that irradiation on 8 consecutive days with solar-simulated UV induced a heavier epidermal increase at the caudal end of the mouse dorsum as compared with the cranial end of the dorsum, whereas a stationary level was reached for both anatomical locations after 22 times of UV irradiation.

Laser treatment of vascular lesions is optimally based on a relatively unimpeded passage of laser light through the epidermal layers, followed by selective energy deposition to the targeted, intravascular hemoglobin and confinement of thermal damage to the dilated dermal vessels, thus allowing a safe and effective clearing of the vascular lesion with a low occurrence of scarring and permanent pigmentary changes. However, before targeting the intravascular hemoglobin, the laser light may be weakened either by absorption in the overlaying melanin, by attenuation in the epi-

dermal layers, or by scattering effects by dermal, collagen fibres.

In the normal epidermis, melanin is mainly responsible for attenuation of wavelengths in the visible spectrum, the degree depending on skin type, whereas the importance of epidermal thickness decreases with increasing wavelength [21–23]. Our results, nevertheless, demonstrated that increased epidermal thickness in hairless, albino mice did reduce acute skin reactions and chronic side effects from treatment with 578 nm wavelength, which is supposed to be due to an increased epidermal attenuation of laser light, thus allowing less laser energy to reach the underlying dermal vessels. The possibility could not be excluded, however, that UV—induced variations in the vascular bed might contribute to our results, and we therefore evaluated the histological appearance of the vessels by means of an indirect antigen-antistof technique for factor VIII related antigen, staining for endothelium. Neither quantitative nor qualitative differences could be detected, and accordingly we consider histological variations in vessels of minor importance for our results. In contrast, it could be that our results partly are to be explained by UV impairment of the skin immune response as described by Kripke and others [24]. However, we do not consider this immune suppression to be of major importance to our results, since the suppression lasts for only 3 days after the final UV irradiation [25], and in our study 4 days passed between UV irradiation and laser treatment. In the present study we found that ~ 25% higher laser fluences had to be applied to skin preirradiated with 22 times of UV compared with nonirradiated skin in order to induce the same score of fibrosis (score 1), and consequently, it may be necessary to use intensified laser fluences for treatment of vascular lesions, which are covered with a thick epidermis. Application of 25% intensified laser fluences, however, may result in an important increased risk of inducing side effects, as we in a previous study estimated that normal skinned persons of a fair complexion increased their risk of getting pigmentary changes ~ 40% and their risk of getting scar formation ~ 10%, when laser intensities were increased 43% from 0.7 W to 1.0 W (26). This leaves the 25% intensified fluences to result in a 23% higher risk of inducing pigmentary changes and a 6% higher risk of inducing scarring. We, therefore, assume that CVL treatment of vascular lesions in skin areas with a thick overlaying epidermis are at risk of getting side-effects, which may be due to heat generation in the epidermal layers and subsequent heat conduction to dermis.

Increased skin thickness has, among other factors, been suggested as an explanatory variable that might account for anatomical- and age variations in response to treatment with the pulsed dye laser. Sonography has shown that skin thickness displays anatomical differences and increases linearly with age until 20 years [27,28], and it is well known that the anatomical location of PWS is important to the clinical outcome, since PWS on trunk, upper and lower extremities respond less favourably than PWS located on face and neck [17,29] and since centrofacial lesions and lesions involving dermatome V_2 respond less favourably than lesions located elsewhere on the head and neck [18]. Furthermore, it is well known that younger patients with light pink, macular PWS require fewer treatments with the pulsed dye laser to obtain the same degree of lesion lightening as older patients with darker, nodular lesions [29–31]. However, no clear association has been established between clinical results from laser treatment and skin thickness, pointing out that other factors, such as lesion color, microstructure of skin, or depth of vessels in the vascular lesion, may contribute to differences in efficacy of the dye laser [16,17]. Another problem is that sonography evaluates the thickness of epidermis and dermis in combination and therefore cannot be directly compared with epidermal thickness [32]. Moreover, the above mentioned observations concerning age and anatomical variations in response to treatment with the pulsed dye laser cannot be directly transferred to our results with the CVL, since the pulsed dye laser, contrary to the CVL, achieves selective photothermolysis and since the depth to which light penetrates skin is directly proportional with its wave-length, allowing the pulsed dye laser to penetrate to deeper components of the vascular lesion as compared with the CVL [22].

Our results suggest that UV-induced epidermal thickening may reduce the efficacy of CVL—treatment after sun exposure, not solely due to an increased content of the competitive chromophore, melanin, but also due to increased epidermal thickness, which attenuates the laser light before reaching the targeted dermal vessels. Furthermore, our results indicate that variations in epidermal thickness might contribute to an explanation of regional and age differences in response to laser treatment.

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